Muscle fatigue resistance during stimulated contractions is reduced in young male smokers.

Morse, C.I.; Wust, R.C.I.; Jones, D.A.; de Haan, A.; Degens, H.

published in
Acta Physiologica
2007

DOI (link to publisher)
10.1111/j.1748-1716.2007.01721.x

document version
Publisher's PDF, also known as Version of record

Acta Physiol 2007, **191**, 123–129

Muscle fatigue resistance during stimulated contractions is reduced in young male smokers

C. I. Morse,[1] R. C. I. Wüst,[1] D. A. Jones,[1,2] A. de Haan[1,3] and H. Degens[1]

1 *Institute for Biophysical and Clinical Research into Human Movement (IRM), Manchester Metropolitan University, Cheshire, UK*

2 *School of Sport and Exercise Sciences, the University of Birmingham, Birmingham, UK*

3 *Institute for Fundamental and Clinical Homan Movement Sciences, Faculty of Human Movement Sciences, Vrije University, Amsterdam, the Netherlands*

Received 2 January 2007, revision requested 11 March 2007, revision received 14 March 2007, accepted 11 April 2007
Correspondence: C. I. Morse, Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Alsager Campus, Hassall Road, Alsager, Cheshire ST7 2HL, UK.
E-mail: c.morse@mmu.ac.uk

Abstract

Aim: To determine whether muscle function is compromised in healthy smokers in comparison with activity-matched non-smokers.

Methods: Nine male smokers (aged 22.2 ± 2.5 years: mean ± SD) with a smoking history of 2.5 ± 3.1 pack years, and ten male control participants (25.4 ± 2.9 years) matched for physical activity level participated in this study. Knee extensor strength was measured using isometric maximal voluntary contractions. Voluntary activation of the quadriceps and co-activation of the biceps femoris were determined using interpolated twitches and surface electromyography respectively. The frequency–torque relationship and fatigue resistance were assessed with electrically evoked contractions. A fatigue index was determined as the ratio of final torque to initial torque during a series of isometric contractions (2 min; 30 Hz; 1 s contraction/1 s rest). Quadriceps anatomical cross sectional area was measured with MRI at 50% of femur length.

Results: Maximal voluntary contraction torque, quadriceps anatomical cross sectional area, knee extensor torque/quadriceps cross sectional area, activation, co-activation and force–frequency relationship were similar, whereas the fatigue index was 17% lower in smokers than non-smokers.

Conclusion: In young men smoking does not significantly affect quadriceps muscle mass and contractile properties, but does reduce fatigue resistance of the quadriceps muscle, which was not attributable to differences in physical activity.

Keywords contractile properties, electrical stimulation, isometric strength, quadriceps cross-sectional area, smoking.

Smoking is a recognized risk factor for cardiovascular disease, lung cancer and chronic obstructive pulmonary disease (COPD). Many patients with COPD and chronic heart failure suffer from muscle dysfunction, which is not directly related to changes in lung or heart function (Gosker *et al.* 2000). Besides muscle wasting, which results in weakness (Franssen *et al.* 2005), there is a reduction in the oxidative capacity (Gosker *et al.* 2000) and a shift towards faster fibre types (Satta *et al.* 1997) which may explain the reduced muscle endurance in COPD patients (Van't Hul *et al.* 2004).

In addition to damaging the lungs, smoking may also affect skeletal muscle (Agusti *et al.* 2003, Gan *et al.* 2004, 2005). Smoking has been reported to cause a decline in muscle strength (Örlander *et al.* 1979, Al-Obaidi *et al.* 2004). As muscle cross-sectional area is the major determinant of strength (Maughan *et al.* 1983) it is likely that the lower muscle strength in smokers is attributable to a smaller muscle mass.

 doi: 10.1111/j.1748-1716.2007.01721.x

However, where differences in muscle strength have been reported between smokers and non-smokers, muscle size has not been measured. Furthermore, a shift from high-oxidative type 1 (slow-twitch) fibres towards a higher percentage of low-oxidative type II (fast-twitch) fibres and a reduction in mitochondrial volume (Örlander *et al.* 1979) occurs; changes that are likely to result in a reduction of fatigue resistance (Degens & Veerkamp 1994). It has been suggested that the differences in skeletal muscle between smokers and non-smokers is at least partly due to a lower physical activity level of smokers (Örlander *et al.* 1979). It is important to minimize this bias when studying the effects of smoking on skeletal muscle. Indeed, disuse induces the same changes in skeletal muscle. Therefore, it is important to take into account the physical activity level to separate the effects of disuse from those of smoking *per se*.

Thus, the aim of the present investigation was to determine whether (i) smokers are weaker than non-smokers, (ii) any weakness is the result of neural and/or muscular factors, (iii) there is any difference in contractile properties between smokers and non- smokers, and (iv) fatigue resistance is altered in smokers compared to non-smokers. To exclude the role of disuse, control male participants were activity-matched with healthy smokers. To circumvent possible differences in motivation between smokers and non-smokers, electrically evoked muscle contractions were used to test muscle function. Considering the role of smoking in the development of COPD and its potential effects on skeletal muscle the present study may reveal changes in skeletal muscle function of young healthy smokers (i.e. free from any pre-existing medical condition) before any clinical signs of COPD develop.

Methods

Participants

Nine male smokers and ten male non-smokers (Table 1) participated in the study. Smokers and non-smokers were matched for age and physical activity level. Written informed consent was obtained from all participants and all procedures were approved by the Local Ethics Committee of Manchester Metropolitan University. Potential participants who suffered from respiratory, cardiovascular or neuromuscular diseases or had any lower limb injury were excluded from the study. All participants abstained from smoking and caffeine ingestion for 2 h prior to testing. Physical activity (PA) levels were assessed using a previously validated questionnaire (Baecke *et al.* 1982); where 6 indicates a sedentary lifestyle and 12 a high physical activity level. All participants were relatively inactive with a mean score of around 8 for each group (Table 1). Smoking history was assessed by questionnaire and smoking volume was determined by pack-years, which is defined as the number of cigarette packs smoked per day, multiplied by the number of years smoking. The vital capacity (VC) forced expiratory volume (FEV) was assessed with spirometry (Vitalograph Ltd., Bucks, UK) and used to determine the percentage of the age and height adjusted predicted value (FEV_1%pred) (Crapo *et al.* 1981).

Table 1 Participant characteristics

	Smokers ($n = 9$)	Non-smokers ($n = 10$)
Age (years)	22.2 ± 2.5	25.4 ± 2.9
Mass (kg)	79.9 ± 14.0	76.1 ± 8.7
Height (cm)	180.2 ± 6.5	179.9 ± 6.4
BMI (kg m^{-2})	26.6 ± 2.3	23.5 ± 1.5
VC (L)	4.4 ± 0.8	5.0 ± 0.6
FEV_1%pred	91.3 ± 13.7	104.7 ± 9.9
Physical activity	8.3 ± 1.6	8.7 ± 1.5
Smoking history (pack years)	2.5 ± 3.1	–

BMI, body mass index; VC, vital capacity; FEV_1%pred, the percentage of the age and height adjusted predicted FEV_1.

Experimental set-up

Participants were familiarized with the testing procedures on a separate session prior to data collection. All knee extension torque measurements were performed on the right leg with a Cybex norm dynamometer (Ronkonkoma, New York, NY, USA). Participants were seated with the hip joint at 90 ° flexion with the hip and shoulders strapped to prevent any extraneous movement. Before the experiment, participants warmed-up by performing five submaximal isokinetic contractions. Torque was displayed on a computer screen, interfaced with an acquisition system (ACKNOWLEDGE; Biopac Systems, Santa Barbara, CA, USA) used for analogue-to-digital conversion. The sampling frequency was 2000 Hz. Each torque signal was filtered with a low-pass fourth order Butterworth filter with a 30 Hz cut-off frequency.

Isometric maximal voluntary contractions (MVC) torque

The optimal angle for torque generation appeared to be at 80 ° (full knee extension = 0 °) in all but two participants (data not shown). Therefore, to reduce the influence of joint angle on twitch characteristics all subsequent measurements were performed at a knee joint angle of 80 °. MVCs were maintained for 4 s (sufficient to reach a plateau) with 2 min of rest in

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01721.x

between each contraction to prevent development of fatigue. To maximize performance, visual feedback of the torque signal and verbal encouragement was given to all participants. The highest torque reached during a contraction was recorded, and the highest value of the two contractions was given as the MVC.

Voluntary activation

Voluntary activation levels were determined with the interpolated twitch technique as described previously (Allen *et al.* 1995). Briefly, the muscle was stimulated percutaneously, with the anode (76 mm × 127 mm; Versastim, Conmed Corp., New York, NY, USA), placed over the proximal region of the quadriceps and the cathode over the distal third of the femur length. To assess the supra-maximal current, single pulses (pulse width 50 μs; DSV Digitimer Stimulator, Digitimer Ltd., Herts, UK) were administered at 30 s intervals with increases in current of 5–10 mA, until no further increase in torque was observed. For the interpolated twitch technique, a first doublet (pulse width of 50 μs, interpulse interval 10 ms) was applied with the participant in a relaxed state and a second during the plateau phase of the MVC. The ratio of interpolated and resting doublets was used to provide an index of activation (Allen *et al.* 1995) as follows:

$$\text{Activation (\%)} = [1 - (\text{superimposed doublet torque}/\text{resting doublet torque})] \times 100,$$

where the superimposed doublet torque is the additional torque during the MVC caused by the doublet.

Antagonist co-activation

To determine whether antagonist co-contraction affected the measured torque during a knee extensor MVC, the electromyographic (EMG) activity of the biceps femoris was recorded as described previously (Kellis & Baltzopoulos 1997). Two 10-mm, pre-gelled, Ag-AgCl percutaneous unipolar electrodes (Medicotest, Olstykke, Denmark) were placed along the mid sagittal line of the biceps femoris muscle to reduce the level of cross talk. To ensure that EMG recordings were made beyond the motor point of the biceps femoris, electrodes were positioned at a third of total femur length (Zipp 1982), with the reference electrodes placed over the lateral epicondyle of the femur. The raw EMG activity was acquired with a sampling frequency of 2000 Hz and processed with a multi channel analogue–digital converter (Biopac Systems Inc., Santa Barbara, CA, USA). The raw EMG signal was filtered with low and high-band pass filters set at 500 and 10 Hz, respectively, and amplified with a gain of 2000. The level of co-activation of the biceps femoris was assessed using the root mean square (RMS) of the raw EMG signal which was integrated over 1 s about the peak MVC torque during knee extension and was expressed as the percentage of activity recorded from the biceps femoris during maximal knee flexion (Klein *et al.* 2001).

Contractile properties

The frequency–torque relationship and fatigue resistance were determined with electrically evoked contractions essentially as described previously (Degens *et al.* 2005). Briefly, the intensity of stimulation throughout the test was such that about 30% of MVC toque was developed during 100-Hz.

Five minutes after the last MVC, the quadriceps muscle was stimulated with 1, 10, 15, 20, 30, 50 and 100-Hz trains for 1 s, separated by 1 min, to assess the frequency–torque relationship. Frequencies were delivered in a random order. From the 100-Hz tetanus of the frequency–torque relationship, the contraction time was measured as the time from activation until 90% of the maximal torque was reached, and the relaxation time as the time for the torque to decline to 50% of maximal torque after cessation of stimulation (Degens *et al.* 2005).

Fatigue test

Five minutes after the contractions for the frequency–torque relationship the resistance to fatigue was assessed by stimulating the quadriceps muscle with 30-Hz trains, 1-s on 1-s off, for 2 min (Degens *et al.* 2005). Fatigue (FI) was expressed as the torque at the end of the test divided by that at the start of the test.

Anatomical cross-sectional area

Anatomical cross-sectional area (ACSA) of the quadriceps was measured with magnetic resonance imaging (MRI), using a fixed 0.2-T MRI scanner (E-Scan; ESAOTE Biomedica, Genova, Italy), at 50% of femur length. Scans were obtained with a T1 weighted, high resolution, gradient echo profile, with the following scanning parameters: time to echo – 16 ms; repetition time – 100 ms; field of view – 330 mm × 254 mm – matrix: 256 × 256, and a slice thickness of 5 mm. Subjects were supine for 15 min prior to the scan to make sure any fluid shifts had stabilized. To account for differences in muscle mass between the two groups, knee extensor torque was scaled with quadriceps ACSA (Maughan *et al.* 1983) and expressed as Torque/ACSA (Nm cm^{-2}).

Statistical analysis

Data are reported as mean ± SD. To determine the effects of smoking an unpaired two-tailed Student's

 doi: 10.1111/j.1748-1716.2007.01721.x

t-test was performed. None of the parameters violated the assumptions of the *t*-test except for Physical Activity scores which were compared using a non-parametric Mann–Whitney test. Differences were considered significant at $P < 0.05$.

Results

Subject characteristics

Table 1 shows the subject characteristics. Body mass, stature and body mass index (BMI) did not differ between smokers and non-smokers (Table 1). The smokers and non-smokers were successfully matched for physical activity as the PA scores did not differ significantly (Table 1). Smoking history ranged from 0.4–11.1 pack years, with a median of 1.7 years (Table 1). The non-smokers had never smoked. VC and FEV_1%pred did not differ significantly between smokers and non-smokers (Table 1).

Strength and muscle size

Knee extensor MVC torque was not significantly different between smokers and non-smokers (Table 2). This and the similar ACSA were reflected in the absence of a significant difference in the MVC/ACSA between smokers and non-smokers (Table 2). Also, the level of voluntary activation and co-activation of the antagonist muscles during an MVC were not significantly different between smokers and non-smokers (Table 2).

Contractile properties

The contraction times of the 100-Hz tetani were not significantly different between the smokers and non-smokers (Table 2). Similarly, half relaxation time was comparable in the two groups. In line with this the frequency–torque relationship of the quadriceps of smokers and non-smokers showed no difference (Fig. 1).

Table 2 Quadriceps function and contractile characteristics from male smokers and non-smokers

	Smokers ($n = 9$)	Non-smokers ($n = 10$)
MVC (Nm)	335 ± 73	351 ± 72
ACSA (cm^2)	74.9 ± 11.6	78.9 ± 6.7
MVC/ACSA (Nm cm^{-2})	4.5 ± 0.5	4.5 ± 0.9
Activation (%)	92 ± 3	93 ± 9
Co-activation (%)	27 ± 17	18 ± 10
Contraction time (ms)	334 ± 125	293 ± 67
$\frac{1}{2}$ Relaxation time (ms)	112 ± 6	117 ± 8

MVC, maximal voluntary isometric knee extension torque; ACSA, anatomical cross-sectional area; MVC/ACSA, MVC torque relative to ACSA; for further details see the *Methods*.

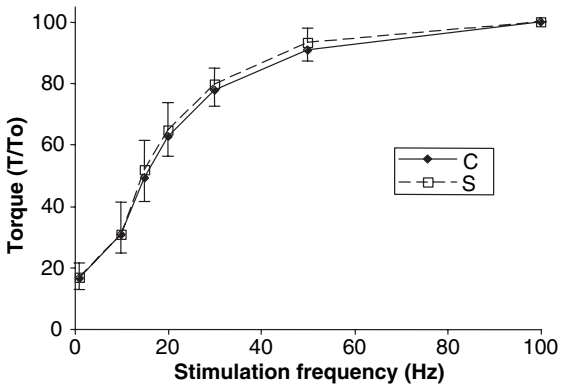

Figure 1 The frequency–torque relationship in healthy young male smokers (S) and non-smokers (C). T/T_0: Torque at a certain frequency as a percentage of the torque at 100 Hz.

Fatigue index

In Figure 2 it can be seen that the quadriceps muscles of the smokers were more susceptible to fatigue than those of the non-smokers ($P < 0.05$); after 2 min of 30-Hz stimulation the FI was 0.54 ± 0.08 vs. 0.62 ± 0.09 in

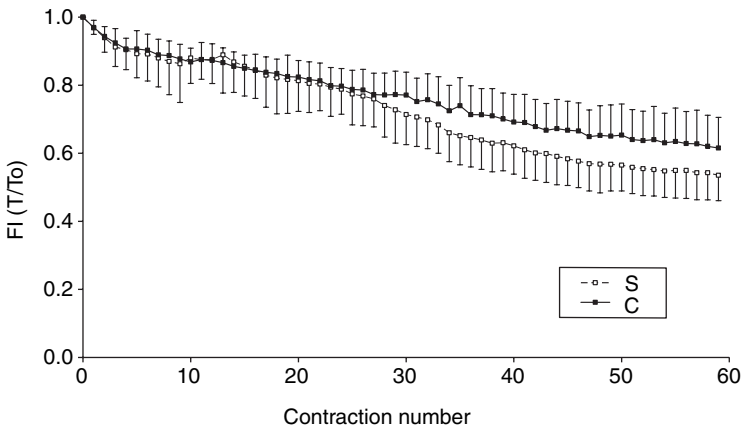

Figure 2 Fatigue resistance of the quadriceps muscle in healthy young male smokers (S) and non-smokers (C). The torque (T/T_0), elicited by electrical stimulation (30-Hz, 1-s on 1-s off) of the quadriceps muscle, is presented as relative to the torque generated during the first contraction. The torque decline was larger in smokers than non-smokers ($P < 0.05$).

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01721.x

smokers and non-smokers respectively. The variation in FI among the smokers did not correlate with either smoking volume in terms of pack years ($R^2 = 0.09$, $P > 0.05$) or FEV_1%pred ($R^2 = 0.28$, $P > 0.05$).

Discussion

The main finding of the present study was a decrease in fatigue resistance in young male smokers compared with a group of physical activity-matched non-smokers, while strength and other contractile properties did not differ between the groups. Based on the present results the decrease in fatigue resistance cannot be explained by disuse, or restricted airflow. The electrically stimulated fatigue test eliminates the possibility that differences in fatiguability are due to differences in motivation and it is probable that the cause of the lower fatigue resistance in smokers is located within the muscle, at or beyond the level of the neuromuscular junction.

Strength

We observed no differences in knee extension strength between smokers and non-smokers. Two other studies, however, have reported a decline in maximal voluntary strength in smokers (Örlander *et al.* 1979, Al-Obaidi *et al.* 2004). Such a decline can, at least theoretically, be a consequence of a smaller muscle mass, a reduced ability to voluntarily activate the muscle and/or increased activation of antagonistic muscles during a maximal contraction. These factors could be secondary to lower levels of activity in the smokers in these previous studies. Indeed, it has been suggested that a lower level of physical activity underlies the decline in strength previously observed in smokers (Örlander *et al.* 1979). In the present study smokers and non-smokers were matched for physical activity level and, similarly to Larsson *et al.* (Larsson 1984), we did not observe a difference in strength between smokers and non-smokers. Thus, the decrease in strength observed in previous studies is likely to be explicable by a reduced activity level in smokers, rather than an effect of smoking *per se* on maximal knee extensor strength or other determinants of strength (activation, co-activation and ACSA).

Fatigue

Although strength was not affected by smoking, the fatigue resistance of the quadriceps muscle was significantly reduced. Others, however, have not observed a change in the fatigue resistance during maximal voluntary contractions in smokers (Örlander *et al.* 1979, Larsson 1984). This discrepancy is difficult to explain but may, in part, be related to the type of fatigue tests performed. Previous studies have used a series of fast dynamic contractions where the recovery interval between contractions was probably around half a second compared to the 1-s interval in the present study. Dynamic contractions are more energetically demanding than the isometric contractions we have used. Consequently previous work may have been testing predominantly anaerobic function whilst our protocol emphasizes more the aerobic capacity of the muscle and the ability to recover between successive contractions.

The acute neural stimulation effect of nicotine in cigarette smoke is well established (Cryer *et al.* 1976) and Mundel & Jones (2006) have demonstrated that nicotine has a beneficial effect on cycling endurance. It might be hypothesised that the stimulant effect of nicotine during voluntary fatigue tests may counteract some peripheral mechanism which contributed to the greater levels of fatigue in the smokers in the present study.

Smoking is the primary cause of COPD, which is characterized by a decline in FEV_1, and it has been reported that smoking itself causes an increase in pulmonary airway resistance (Nadel & Comroe 1961). However, we did not observe a significantly lower FEV_1%pred in smokers than non-smokers, or any correlation between fatigue resistance and FEV_1%pred. Therefore it is unlikely that lung function and the ability to oxygenate the blood play any role in the lower fatigue resistance of smokers. Indeed, as the fatigue test was performed on one leg, with activation of only about 30% of the quadriceps, blood oxygenation is unlikely to be a limiting factor.

Lower levels of habitual physical activity level might lead to reduced oxidative capacity but in the present investigation the similar activity levels suggest that it is cigarette smoking itself which is responsible for the observed reduction in fatigue resistance.

A number of mechanisms (both acute and chronic) may be responsible for the observed decline in fatigue resistance, these include alterations in muscle fibre type composition and/or a decreased oxidative capacity (Örlander *et al.* 1979, Larsson 1984).

Although the frequency–torque relationship and contraction and relaxation times are not unequivocal indicators for changes in fibre type composition, the absence of significant alterations in these parameters indicates no major changes in fibre type composition.

Mitochondrial enzyme activities (citrate synthase and 3-hydroxyacyl-CoA dehydrogenase) have been reported to be decreased in the muscles of smokers (Örlander *et al.* 1979). Interestingly, healthy ex-smokers did not show a decline in activities of enzymes of oxidative metabolism (Larsson 1984) and COPD patients who stopped smoking exhibited normal fatigue resistance (Degens *et al.* 2005). This suggests that the smoking-

 doi: 10.1111/j.1748-1716.2007.01721.x

induced alterations in the activity of oxidative enzymes and muscle fatigue resistance are reversible. Furthermore, the fact that we found no relationship between pack years and fatigue resistance suggests an acute effect of smoking. Cyanide and carbon monoxide (CO), in smoke are possible candidates for acutely affecting fatigue resistance. Cyanide and CO inhibit cytochrome *c* oxidase (complex IV in the mitochondrial chain), causing an overall decline in mitochondrial function and oxidative capacity (Alonso *et al.* 2003), and thus potentially a reduced fatigue resistance of the muscle.

Beside acting on cytochrome *c* oxidase, CO in cigarette smoke increases Carboxyhemoglobin (COHb) levels (Puente-Maestu *et al.* 1998), which reduces the O_2 carrying capacity of the blood. However, a reduced O_2 carrying capacity of the blood induced by breathing hypoxic air had no effect on fatigue resistance during the same test as employed in the present study (Degens *et al.* 2006). COHb also hampers the release of O_2 as reflected by the left-ward shift of the O_2-Hb binding curve. Furthermore, the facilitated diffusion of O_2 by myoglobin within the muscle may be impaired by the formation of carboxymyoglobin (Wittenberg & Wittenberg 1987). It has been reported that raised levels of COHb reduce O_2 extraction during stimulated contractions with *in situ* canine gastrocnemius, which was accompanied by a reduction in muscle tension (King *et al.* 1987).

Besides changes in the muscle itself, another possibility for the earlier onset of fatigue in smokers might be related to neurotransmission failure (Gandevia 2001). Using direct muscle stimulation superimposed on nerve stimulation, it has been shown that neurotransmission failure contributes to an earlier onset of fatigue during hypoxia (Zhu *et al.* 2006). With the present stimulation protocol we are unable to state whether neural transmission failure contributed to the earlier onset of fatigue in smokers.

We assume that the more rapid fatigue, whatever the cause, is a consequence of changes occurring as a result of smoking. However, we cannot exclude the possibility that there may be some genetic component that, for instance, influences the likelihood of smoking and, at the same time, influences fatigue properties of the muscle.

The relationship between the changes in fatigue resistance we report here and the muscle dysfunction frequently seen in COPD patients is unclear. Given that fatigue resistance is not affected in COPD patients who stopped smoking (Degens *et al.* 2005) and the fact that we saw no association between pack years smoked and FI, it is likely that the reduced fatigue resistance in the present smokers is acute in nature. If it is an acute response, the underlying factors of this fatigue are most likely changes in the oxidative capacity of the muscle brought about, for instance, by a reversible effect of cyanide and/or CO on mitochondrial enzyme function and tissue oxygenation. Indeed, 28 days of smoking cessation has been shown to result in a return to normal mitochondrial respiratory chain function (Cardellach *et al.* 2003). Therefore, future studies may be directed towards the acute effects of CO, smoking and smoking cessation on skeletal muscle function, oxygenation and fatigue resistance.

Conflict of interest

There is no conflict of interest.

References

Agusti, A.G., Noguera, A., Sauleda, J., Sala, E., Pons, J. & Busquets, X. 2003. Systemic effects of chronic obstructive pulmonary disease. *Eur Respir J* **21**, 347–360.

Al-Obaidi, S.M., Anthony, J., Al-Shuwai, N. & Dean, E. 2004. Differences in back extensor strength between smokers and nonsmokers with and without low back pain. *J Orthop Sports Phys Ther* **34**, 254–256.

Allen, G.M., Gandevia, S.C. & McKenzie, D.K. 1995. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve* **18**, 593–600.

Alonso, J.R., Cardellach, F., Lopez, S., Casademont, J. & Miro, O. 2003. Carbon monoxide specifically inhibits cytochrome c oxidase of human mitochondrial respiratory chain. *Pharmacol Toxicol* **93**, 142–146.

Baecke, J.A., Burema, J. & Frijters, J.E. 1982. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* **36**, 936–942.

Cardellach, F., Alonso, J.R., Lopez, S., Casademont, J. & Miro, O. 2003. Effect of smoking cessation on mitochondrial respiratory chain function. *J Toxicol Clin Toxicol* **41**, 223–228.

Crapo, R.O., Morris, A.H. & Gardner, R.M. 1981. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* **123**, 659–664.

Cryer, P.E., Haymond, M.W., Santiago, J.V. & Shah, S.D. 1976. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N Engl J Med* **295**, 573–577.

Degens, H. & Veerkamp, J.H. 1994. Changes in oxidative capacity and fatigue resistance in skeletal muscle. *Int J Biochem* **26**, 871–878.

Degens, H., Sanchez Horneros, J.M., Heijdra, Y.F., Dekhuijzen, P.N. & Hopman, M.T. 2005. Skeletal muscle contractility is preserved in COPD patients with normal fat-free mass. *Acta Physiol Scand* **184**, 235–242.

Degens, H., Horneros, J.M. & Hopman, M.T. 2006. Acute hypoxia limits endurance but does not affect muscle contractile properties. *Muscle Nerve* **33**, 532–537.

Franssen, F.M., Broekhuizen, R., Janssen, P.P., Wouters, E.F. & Schols, A.M. 2005. Limb muscle dysfunction in COPD:

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01721.x

effects of muscle wasting and exercise training. *Med Sci Sports Exerc* **37**, 2–9.

Gan, W.Q., Man, S.F., Senthilselvan, A. & Sin, D.D. 2004. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax* **59**, 574–580.

Gan, W.Q., Man, S.F. & Sin, D.D. 2005. The interactions between cigarette smoking and reduced lung function on systemic inflammation. *Chest* **127**, 558–564.

Gandevia, S.C. 2001. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81**, 1725–1789.

Gosker, H.R., Wouters, E.F., van der Vusse, G.J. & Schols, A.M. 2000. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. *Am J Clin Nutr* **71**, 1033–1047.

Kellis, E. & Baltzopoulos, V. 1997. The effects of antagonist moment on the resultant knee joint moment during isokinetic testing of the knee extensors. *Eur J Appl Physiol Occup Physiol* **76**, 253–259.

King, C.E., Dodd, S.L. & Cain, S.M. 1987. O2 delivery to contracting muscle during hypoxic or CO hypoxia. *J Appl Physiol* **63**, 726–732.

Klein, C.S., Rice, C.L. & Marsh, G.D. 2001. Normalized force, activation, and coactivation in the arm muscles of young and old men. *J Appl Physiol* **91**, 1341–1349.

Larsson, L.O.J. 1984. Skeletal muscle morphology, metabolism and function in smokers and non-smokers. A study on smoking-discordant monozygous twins. *Acta Physiol Scand* **120**, 343–352.

Maughan, R.J., Watson, J.S. & Weir, J. 1983. Strength and cross-sectional area of human skeletal muscle. *J Physiol* **338**, 37–49.

Mundel, T. & Jones, D.A. 2006. Effect of transdermal nicotine administration on exercise endurance in men. *Exp Physiol* **91**, 705–713.

Nadel, J.A. & Comroe J.H. Jr, 1961. Acute effects of inhalation of cigarette smoke on airway conductance. *J Appl Physiol* **16**, 713–716.

Örlander, J., Kiessling, K.H. & Larsson, L. 1979. Skeletal muscle metabolism, morphology and function in sedentary smokers and nonsmokers. *Acta Physiol Scand* **107**, 39–46.

Puente-Maestu, L., Bahonza, N., Perez, M.C., Ruiz de Ona, J.M., Rodriguez Hermosa, J.L. & Tatay, E. 1998. Relationship between tobacco smoke exposure and the concentrations of carboxyhemoglobin and hemoglobin. *Arch Bronconeumol* **34**, 339–343.

Satta, A., Migliori, G.B., Spanevello, A. *et al.* 1997. Fibre types in skeletal muscles of chronic obstructive pulmonary disease patients related to respiratory function and exercise tolerance. *Eur Respir J* **10**, 2853–2860.

Van't Hul, A., Harlaar, J., Gosselink, R., Hollander, P., Postmus, P. & Kwakkel, G. 2004. Quadriceps muscle endurance in patients with chronic obstructive pulmonary disease. *Muscle Nerve* **29**, 267–274.

Wittenberg, B.A. & Wittenberg, J.B. 1987. Myoglobin-mediated oxygen delivery to mitochondria of isolated cardiac myocytes. *Proc Natl Acad Sci USA* **84**, 7503–7507.

Zhu, X., Heunks, L.M., Ennen, L., Machiels, H.A., Van Der Heijden, H.F. & Dekhuijzen, P.N. 2006. Nitric oxide modulates neuromuscular transmission during hypoxia in rat diaphragm. *Muscle Nerve* **33**, 104–112.

Zipp, P. 1982. Recommendations for the standardization of lead positions in surface electromyography. *Eur J Appl Physiol* **50**, 41–54.

